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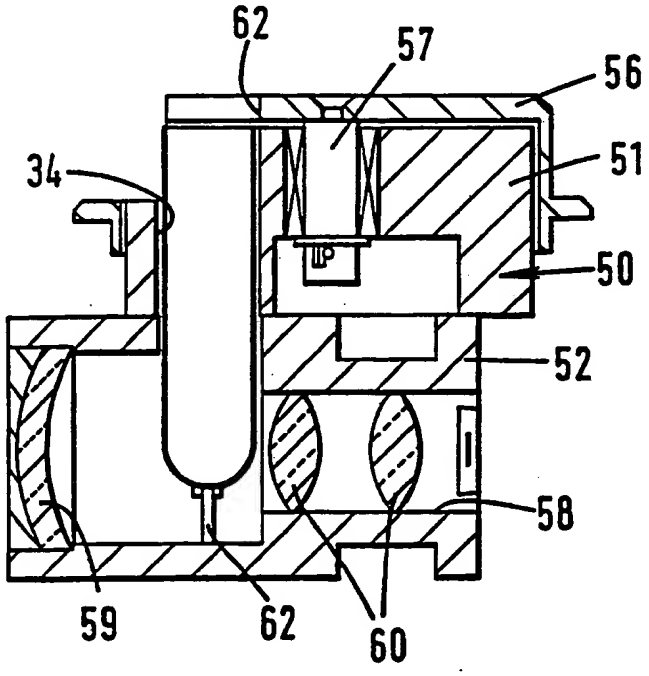
(54) Title: LUMINESCENCE OR FLUORESCENCE MONITORING APPARATUS AND METHOD

(57) Abstract

A monitor for use in sample testing comprises a sample chamber for receiving a vessel containing a light-emitting substance, and an avalanche photodiode for receiving the emitted light and connected to a circuit for measuring the light received by the avalanche photodiode. The use of an avalanche photodiode enables the monitor to be portable and robust and to be included in a portable kit for carrying out hygiene monitoring in the field.



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Luminescence or Fluorescence Monitoring Apparatus and Method

This invention relates to an apparatus and method for monitoring luminescent or fluorescent light emission, and particularly but not solely for use in hygiene monitoring.

It is known to test for bacteria or other living cells by taking a sample and releasing adenosine triphosphate (ATP) contained within the cells using an appropriate reagent together with the enzyme luciferase in order to cause the emission of light. The light output is related to the concentration of ATP in the sample. The light output is however very small and hitherto the light detecting apparatus has made use of a photomultiplier tube in order to provide a measurable electrical signal output. Photomultipliers require a high voltage power supply, normally only available in the laboratory. Hitherto testing for living cells has been carried out by taking a sample in the field, then analysing the sample at a central laboratory either by conventional culture techniques using plate counts, or using luminescence monitoring apparatus with which the laboratory is equipped, together with a power supply appropriate for the photomultiplier tube of that apparatus.

In accordance with this invention, there is provided a monitor for use in sample testing, comprising a sample chamber for receiving a vessel containing a light-emitting substance and a photodetector for receiving the emitted light and connected to an electrical circuit for measuring the light received by the photodetector, in which the photodetector comprises an avalanche photodiode.

The avalanche photodiode is a semi-conductor device which is extremely light-sensitive and responds to a low level of received light to provide a measurable electrical output signal. The circuitry for the avalanche photodiode does not require as high a voltage or as high a current power supply as photomultipliers, and can be powered by batteries. Furthermore the avalanche photodiode is, in contrast with photomultiplier,

robust and not adversely affected by exposure to intense light. Thus the monitor in accordance with this invention can be a portable device.

The monitor in accordance with this invention may be used for measuring the light emitted by substances spontaneously, and it may be used for measuring the light emitted by substances once excited by exposure to light (i.e. fluorescent substances) and references in this specification to a luminescence monitor (or to a light-emitting substance) are intended to include a monitor measuring light produced by fluorescence (or to a fluorescent substance).

Also in accordance with this invention, there is provided a monitor for use in sample testing, comprising a sample chamber for receiving a vessel containing a light-emitting substance, the chamber being formed in its side wall with a light outlet for passing light to a photodetector, and means for adjusting the vertical position of the vessel relative to said light outlet.

The vertical adjustment enables the sample containing vessel to be positioned such that the centre of its sample of e.g. liquid is aligned with the outlet which is passing light to the photodetector. Thus a relatively small sample may be taken and contained in the vessel, yet it can be ensured that the photodetector receives the maximum of the light emitted by the sample.

One use of the monitor is for hygiene monitoring. In accordance with this invention there is provided a method of hygiene monitoring, comprising wiping a swab over a surface to be tested, placing the swab in a pipette tip, coupling a pipette to the pipette tip and using the pipette to draw a quantity of breakage reagent into the pipette, and subsequently expelling the breakage reagent from the pipette tip and into a sample vessel which already contains an enzyme, such that if the swab sample included bacteria or other living cells, light is now emitted from the liquid in the sample vessel.

This hygiene monitoring method is particularly suited to being carried out in the field, using a kit comprising sterile swabs, sterile pipette tips, a container of breakage reagent, sample vessels already charged with enzyme, and a portable luminescence monitor. These items can all be carried in a carrying case.

Thus also in accordance with this invention, there is provided a kit for use in hygiene monitoring comprising a portable carrying case containing a luminescence monitor, a plurality of swabs, a plurality of pipette tips, a pipette for coupling to each pipette tip, a plurality of sample vessels pre-charged with enzyme, and a container of breakage reagent. Preferably also a holder is provided for gripping each swab.

The monitor described above may be used for a wide variety of other sample testing applications, for example testing raw and final products in the food industry, pharmaceutial preparations, water quality and clinical samples. In these cases, a modified monitoring kit is appropriate.

Therefore further in accordance with this invention, there is provided a kit for use in sample testing, comprising a portable carrying case containing a luminescence monitor, a plurality of pipette tips, a pipette for coupling to each pipette tip, a plurality of sample vessels, and containers of breakage reagent and enzyme.

In use of this kit, the sample to be tested is introduced into a sample vessel and then using the pipette with a tip coupled to it, appropriate quantities of the breakage reagent and of the enzyme are also introduced into the sample vessel. The sample vessel is finally placed in the luminescence monitor to measure the light emission.

Embodiments of this invention will now be desribed by way of examples only and with reference to the accompanying drawings, in which:

FIGURE 1 is a plan view of a hygiene monitoring kit shown with its carrying case open;

FIGURE 2 is a side view of the kit shown in Figure 1;

FIGURE 3 is an enlarged plan view of a reagent kit of the hygiene monitoring kit;

FIGURE 4 is a section through a pipette tip when receiving a swab from a swab holder;

FIGURE 5 is a section through the pipette tip when engaged with a pipette;

FIGURE 6 is a section through one form of sample chamber of a portable luminescence monitor of the kit;

FIGURE 7 is a section through another form of sample chamber;

FIGURE 8 is a section through a further form of sample chamber;

FIGURE 9 is a plan view of the sample chamber shown in Figure 8;

FIGURE 10 is a block diagram of the circuit of the monitor, and

FIGURE 11 is a plan view of another embodiment of sample testing kit shown with its carrying case open.

Referring to Figures 1 and 2, a portable hygiene monitoring kit comprises a carrying case having upper and lower halves 10, 12 which are hinged to each other at 11. When closed together and fastened by means of integral catches, the case may be carried by means of a handle 13. When opened up as shown, the two halves of the case lie flat on an e.g. table or benchtop. In the interior of the case, each half receives a number of items or components of the kit within recesses formed in inserts 14, 15 of plastics foam cushioning material. In the lower half 12 of the casing, there is disposed a portable luminescence monitor 16, a reagent kit 17 and a pack of sterile stencils 18. The luminescence monitor 16 may be powered by batteries housed within its own casing, but additionally or instead a power pack 19 may be provided. In the upper half 10 of the case, there is disposed a swab holder 20 and a pipette 21. Further a pipette holder 22 is mounted in the upper half 10 of the case and is movable

between a first position in which it is laid flat int a recess in the foam insert 14 and an upright position as shown in Figur 2.

Referring to Figure 3, the reagent kit 17 comprises a plurality of cuvettes 24, a plurality of strips of sterile swabs 25, a plurality of sterile pipette tips 26 and a bottle of liquid extractant 27. Each of the cuvettes 24 contains a correct quantity of appropriate enzyme, either powdered or freeze dried onto the inner surface of the cuvette, and the cuvette is closed by a cap.

As shown in Figure 4, each swab comprises a short stem 28 provided with an absorbent head 29. Each pipette tip has a barrel portion 30, a tapering lower end 31 and an upper end shaped to fit onto the lower end of the pipette.

In use of the hygiene monitoring kit, a swab is taken out of its individual packaging, and gripped in the lower end of the swab holder 20. The latter has a tubular lower end 32 which is split to form gripping fingers, which are caused to open up when an actuator at the upper end of the swab holder is depressed (so that the gripping end 32 may be engaged over the free end of the swab stem), and which will close up again (to grip the swab stem) when the actuator is released. Then by holding the swab holder, the user wipes the head 29 of the swab over a surface to be tested. Once the sample has in this manner been taken, the swab is released into a pipette tip, as shown in Figure 4.

Next the pipette 21 is engaged to the pipette tip, as shown in Figure 5. Then the pipette is used to draw up a predetermined volume (say 500µl) of breakage reagent 33 from the extractant bottle 27. The pipette is engaged with the pipette holder 22 and allowed to stand for a predetermined time period (e.g 60 seconds). During this period the breakage reagent breaks into any bacteria or other living cells which have been picked up on the swab during the sampling process, and releases the ATP contained within the cells.

Then the breakage reagent 33 within the pipette tip is

ejected into a cuvette containing the appropriate enzyme. The pipette is used to alternately eject the liquid and draw it up again two or three times, in order to ensure adequate mixing. Having now mixed the enzyme with the breakage reagent, then if there were any living cells on the swab, the ATP will react with the enzyme to commence light emission, the strength of the light emission (number of photons emitted) being related to the concentration of bacteria present. After mixing the breakage reagent into the cuvette, the cuvette is placed in the portable luminescence monitor so that the light output can be measured over a predetermined period of time, to provide a measure of the concentration of bacteria in the sample obtained. It is a feature of the enzyme that a constant light output is produced so that this step is not time dependent.

Referring to Figure 1, the portable luminescence monitor includes a socket 34 in its upper surface, into which the cuvette is placed. The socket is then covered over in light-tight manner. Within the monitor, a photodetector is positioned to receive the light emitted from the liquid within the cuvette. In the example shown, the photodetector comprises an avalanche photodiode. Such a photodiode is extremely light sensitive yet its circuitry requires a low voltage and current power supply, so rendering it suitable for the portable hygiene monitoring system being described. The avalanche photodiode requires an operating temperature of typically +5°C to 0°C and its circuitry is arranged to cool it to this level. The monitor may include a digital display, or an analog display, or a printer or a plotter to provide the measurement data.

Figure 6 shows one embodiment of cuvette receiving chamber of the luminescence monitor. A socket 34 is formed in the upper side of the monitor casing and receives the tubular cuvette 24, to rest on a spring-loaded platform 35. An arm 36 is mounted on a post 37 which is rotatably mounted to the monitor casing, and a plunger 38 depends from the lower side of the arm 36. In use, the cuvette 24 is placed in the socket 34, then the

arm 36 is turned until the plunger 38 lies above the cuvette, and then the arm is depressed until it lies against the upper side f the monitor. In this position, the arm 36 seats onto an O-ring seal 39 on the monitor casing to ensure light-tightness. In depressing the arm 36, the plunger 38 displaces the cuvette downwardly into the socket 34. An O-ring seal 40 on the plunger seals with the inside of the socket 34 to enhance the light-tightness. The cuvette needs to be positioned so that the centre of its small column of liquid is aligned with an orifice 41 in the side of the socket 34, through which the light from the liquid is passed to the detector. Different reactions will require different volumes of liquid, so that they will require different vertical positions for the cuvette for effective light collection. This requirement is achieved by turning an adjusting screw 42 which is threaded through the arm 36 and which carries the plunger 38 at its lower end. The adjusting screw preferably has a number of predetermined settings, each one corresponding to a different reaction liquid volume or protocol, and serving to depress the cuvette to its required level.

Figure 7 shows an alternative arrangement in which the vertical adjustment of the cuvette 24 is achieved by means of an adjusting screw 42 on the underside of the monitor, acting on the platform 35 on which the cuvette stands. In this case, a spring loaded plunger 38 is closed onto the top of the cuvette once the cuvette is placed into the socket 34.

Figures 8 and 9 show a sample chamber for the monitor comprising a body 50 formed with socket 34 for receiving the cuvette.

A top part 51 of the body 50 is circular and receives a rotatable cap 56 which has a peripheral skirt encircling the top part 51 of the body 50. The cap 56 is mounted on a stem 57 which passes through the part 51 and is journalled therein for rotation. The top and skirt of the cap 56 are cut-away over an angular portion as shown at 62. A lower part 52 of the body 50 is formed with a through passage 58 perpendicular to the axis of the

socket 34. At one end of this passage, and to one side of the sample tube or cuvette when receiv d in the socket, there is mounted a concave mirror 59. In the passage 58 on the other side of the cuvette are mounted the lens 60 of an optical system for focussing light from the sample in the cuvette onto the avalanche photodiode APD.

In use of the sample chamber shown in Figures 8 and 9, the cuvette is placed into the socket 34 whilst the cap 56 is in the position shown, i.e. so that the socket 34 is exposed through the cut away 62 of the cap. Then the cap 56 is turned through 180°, to cover the top of the socket 34. The cap 56 and body part 51 are precision-made so that the sample chamber is light-tight when closed. A microswitch is included in the body part 51 and closes as the cap reaches its closed position, to turn on the counter of the APD circuit for a predetermined time, e.g. 10 seconds, as will be described with reference to Figure 10.

The cuvette rests with its bottom on a stem 62. Provision for vertical adjustment of the cuvette may be made by mounting this stem 62 to extend through the bottom of the body part 52 along its axis. This adjusting stem may be displaced in the vertical direction by a manually operable screw such as shown in Figure 7. The provision for vertical adjustment of the cuvette may also be used to allow for different height cuvettes, which are typically 47mm or 55mm in height.

Referring to Figure 10, there is shown the circuit in which the avalanche photodiode APD is connected. The circuit comprises a temperature stabilising system for the avalanche photodiode, comprising a Peltier effect heat pump HP mounted adjacent the APD and supplied with current from a temperature control circuit TC for cooling the APD. A temperature sensing thermistor TS is also mounted adjacent the APD and controls via the circuit TC the magnitude of the current supplied to the Peltier heat pump HP. The output signal from the APD is passed to a detector circuit DC which includes a comparator for comparing the signal with a threshold and providing a pulse of

predetermined width and amplitude for each signal exceeding the threshold. These pulses are passed to a counter C. The circuit further comprises a control logic circuit CL to which the microswitch MS in the sample chamber is connected: when the chamber closes and so closes the microswitch, a 10 second timer in the control logic CL is started and the counter C is enabled for the corresponding time interval. At the end of this, the count is displayed on the numerical display ND.

The circuit of Figure 10 further includes a supply circuit PSC powered from rechargeable batteries B and providing the required supplies for the control logic, temperature control, detector, counter and display circuits. The circuit of Figure 10 may be connected to an external power supply unit PSU for operation and/or for recharging the batteries B.

Referring to Figure 11, there is shown another embodiment of portable hygiene monitoring kit comprising a carrying case having upper and lower halves 80,82 hinged to each other at 81, and which can be closed together and fastened by means of integral catches so that the case can be carried by means of a handle 83. When opened up as shown, the two halves of the case lie flat. In the inside of each half of the case, there is a piece of plastics foam cushioning material 84,85 formed with recesses in which the various components of the kit are received. In the lower half there is disposed the portable luminescence monitor 86 having a sample chamber 88 of the type shown in Figures 8 and 9, an on/off switch 90 and a visual display 92. The monitor includes batteries to power its circuits but a power supply unit PSU is also provided together with electrical leads, for connecting the monitor to the mains for powering it and/or recharging its batteries. A pack of individually packaged sterile swabs is provided at 94 and sterile pipette tips are provided at 96. A rack 98 is also provided, including a number of cuvettes 100, and bottles of breakage reagent and enzyme 102, 104. In the upper half of the case, two or more pipettes 106 are provided.

In use of the kit shown in Figure 11, a swab is wiped

over the surface to be tested, then placed into a cuvette to which predetermined volumes of breakage reagent and then enzyme are added, using one of the pipettes with one of the pipette tips coupled to it. The cuvette is allowed to stand for a predetermined period in the rack 98, and is then placed into the sample chamber and the latter is closed: the count appearing on the display 92 at the end of the integrating period (typically 10 seconds) of the monitor is recorded as a measure of the microbial activity of the sample taken up by the swab.

CLAIMS

1. A monitor for use in sample testing, comprising a sample chamber for receiving a vessel containing a light-emitting substance and a photodetector for receiving the emitted light and connected to an electrical circuit for measuring the light received by the photodetector, in which the photodetector comprises an avalanche photodiode.

2. A monitor as claimed in claim 1, in which the electrical circuit comprises a counter which counts the discrete signals issuing from the avalanche photodiode within a predetermined period of time.

3. A monitor for use in sample testing, comprising a sample chamber for receiving a vessel containing a light-emitting substance, the chamber being formed in a side wall with a light outlet for passing light to a photodetector, and means for adjusting the vertical position of the vessel relative to said light outlet.

4. A monitor as claimed in claim 3, in which the adjusting means comprises an element on which the sample vessel rests, the vertical position of which element is presettable.

5. A monitor as claimed in claim 3, in which the adjusting means comprises a spring-loaded element on which the sample vessel rests, and means for bearing against the top of the sample vessel to depress it through a presettable distance.

6. A method of hygiene monitoring, comprising wiping a swab over a surface to be tested, placing the swab in a pipette tip, coupling a pipette to the pipette tip and using the pipette

to draw a quantity of breakage reagent into the pipette, and subsequently expelling the breakage reagent from the pipette tip and into a sample vessel which already contains an enzyme, such that if the swab sample included bacteria or other living cells, light is now emitted from the liquid in the sample vessel.

7. A kit for use in hygiene monitoring, comprising a portable carrying case containing a luminescence monitor, a plurality of swabs, a plurality of pipette tips, a pipette for coupling to each pipette tip, a plurality of sample vessels pre-charged with enzyme, and a container of breakage reagent.

8. A kit as claimed in claim 7, further comprising a holder for gripping each swab.

9. A kit for use in sample testing, comprising a portable carrying case containing a luminescence monitor, a plurality of pipette tips, a pipette for coupling to each pipette tip, a plurality of sample vessels, and containers of breakage reagent and enzyme.

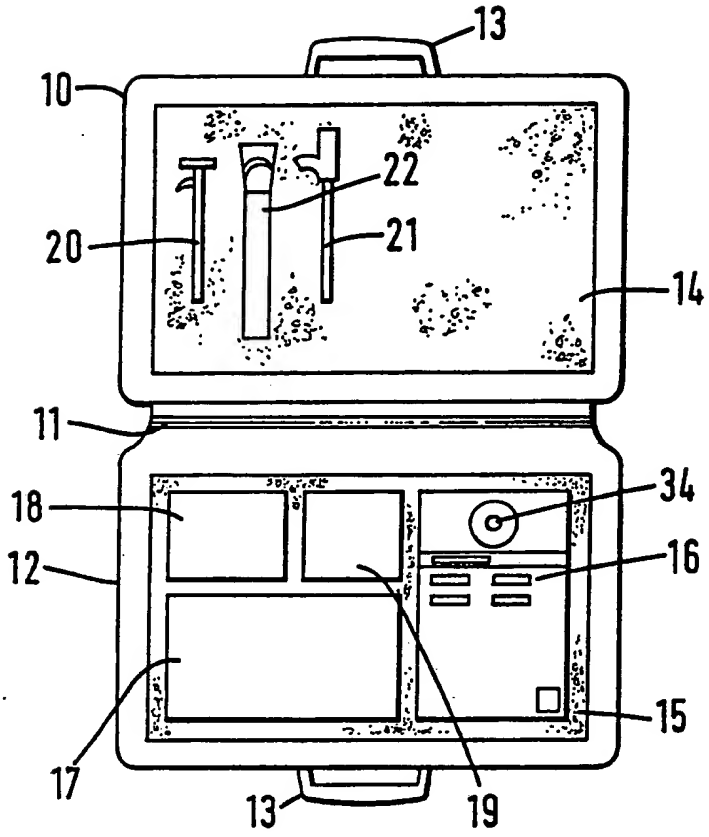


FIG.1

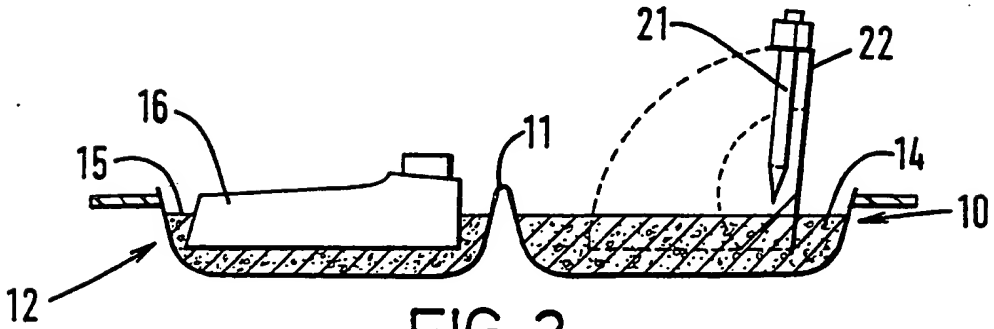


FIG. 2

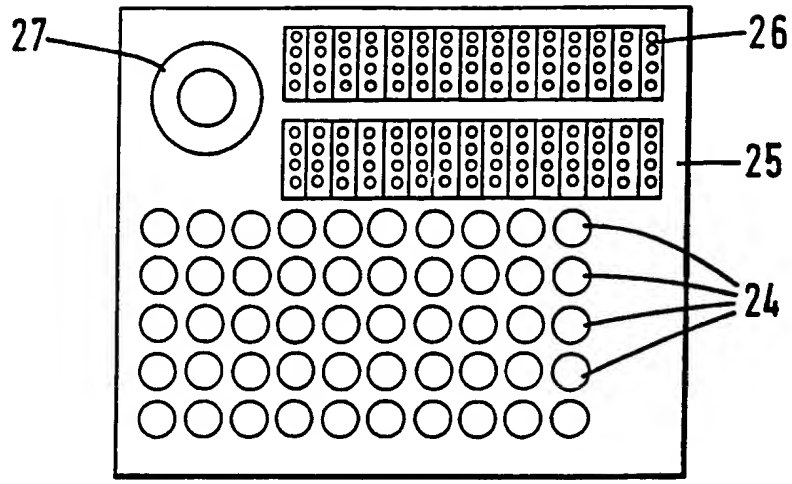


FIG.3

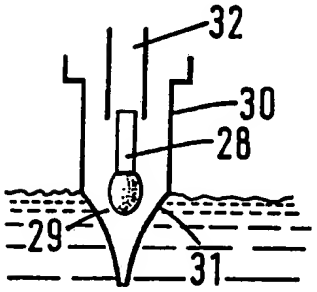


FIG.4

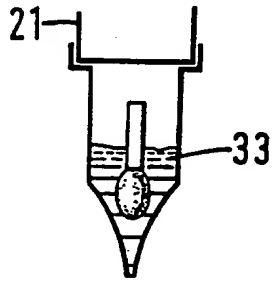


FIG.5

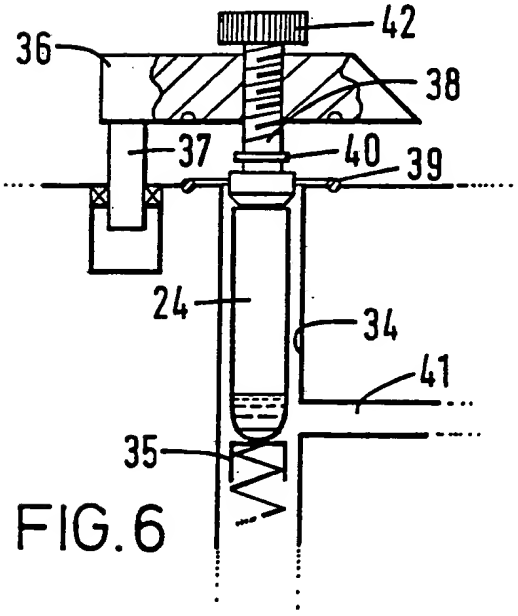


FIG.6

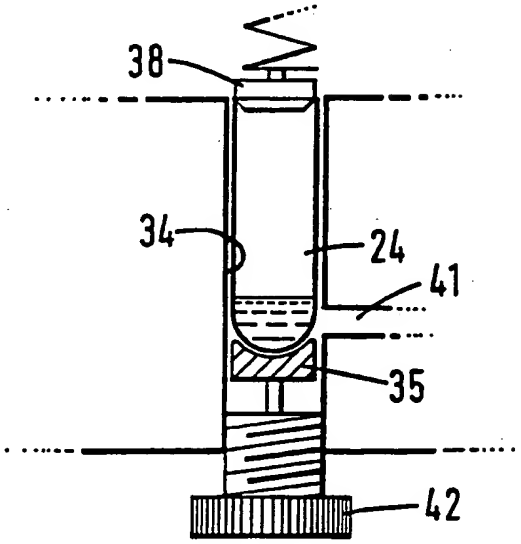


FIG.7

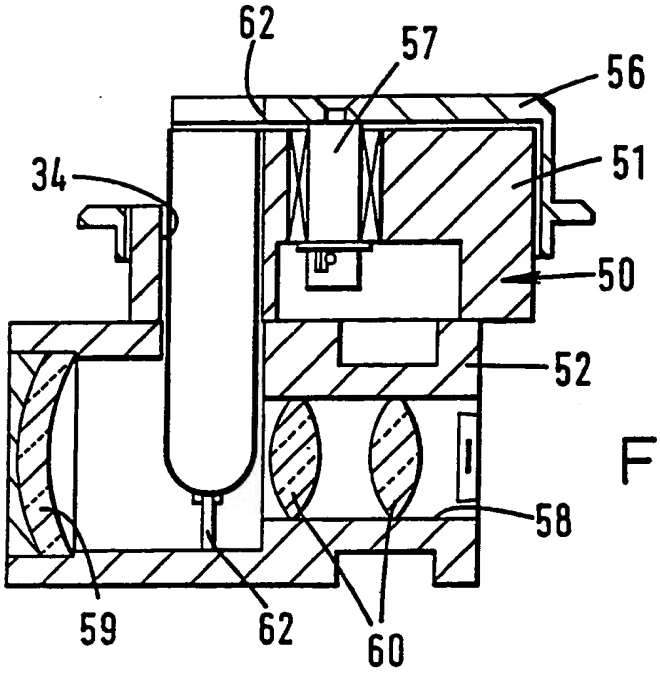


FIG. 8

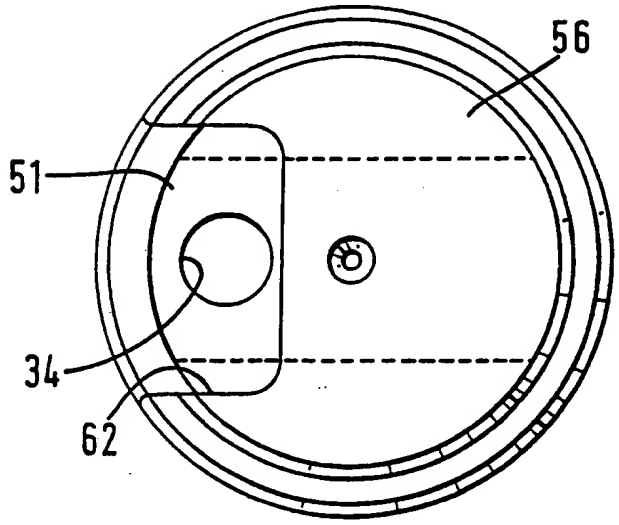
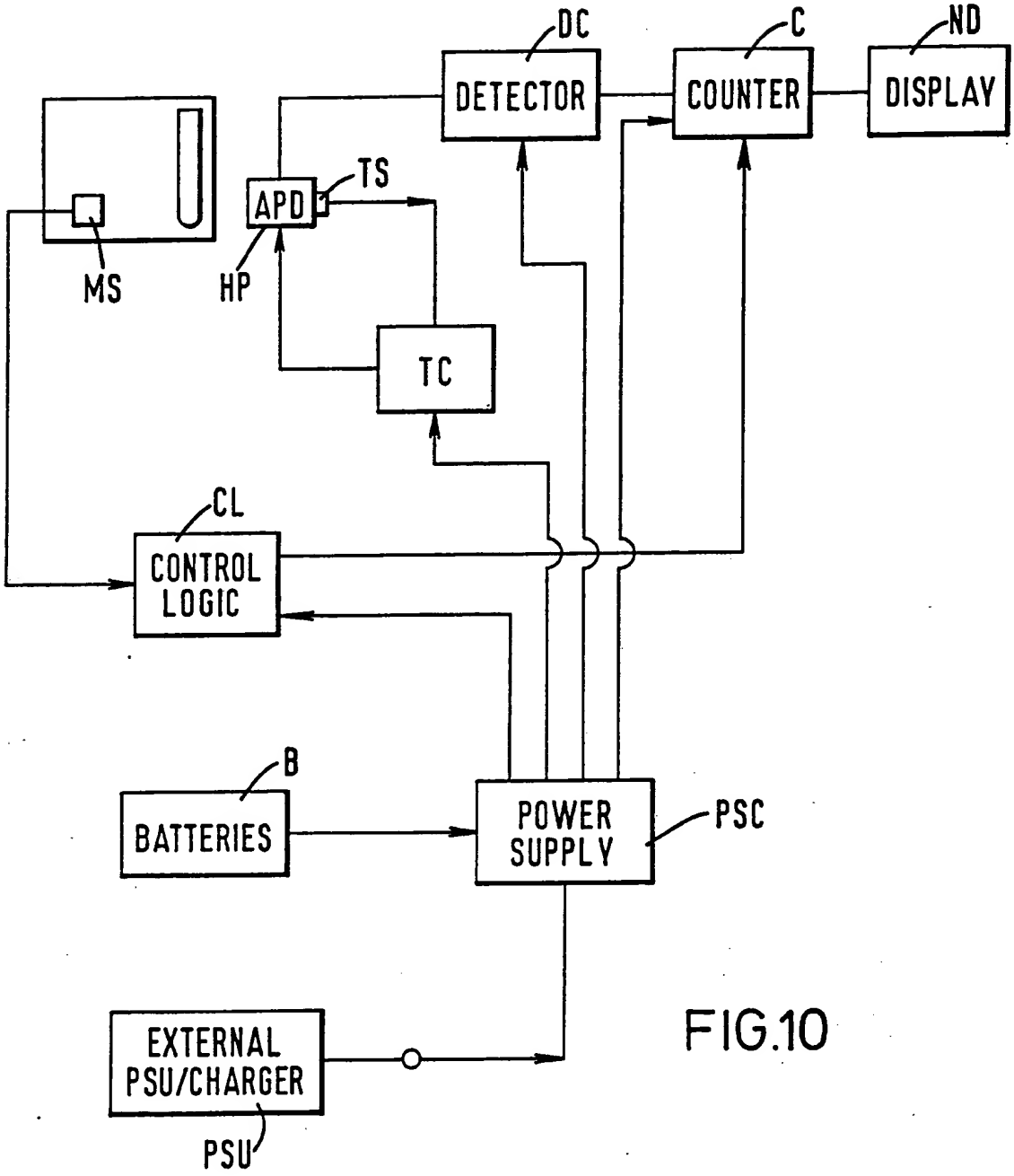


FIG. 9


DC
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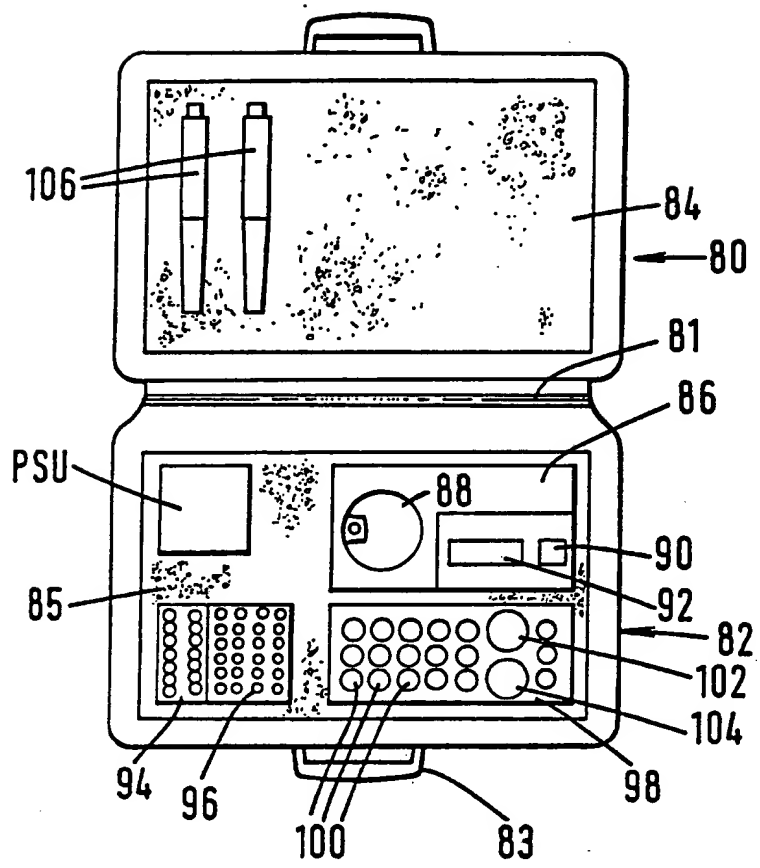
PSC
EXTERNAL PSU/CHARGER
PSU

FIG.10

FIG.11



INTERNATIONAL SEARCH REPORT

International Application No PCT/GB 89/01228

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [6]

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC5: G 01 N 21/76

II. FIELDS SEARCHED

Minimum Documentation Searched [7]

Classification System	Classification Symbols
IPC5	G 01 N

Documentation Searched other than Minimum Documentation to the Extent that such Documents are included in the Fields Searched [8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT [9]

Category [10]	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No. [13]
X	GB, A, 2001434 (SEPPO KOLEHMAINEN) 31 January 1979, see the whole document	3-9
A		1-2
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A	GB, A, 2178847 (PHILIPS ELECTRONIC AND ASSOCIATED INDUSTRIES LIMITED) 18 February 1987, see the whole document	1-9
	--	
A	WO, A1, 85/03518 (AB SANGTEC MEDICAL) 15 August 1985, see the whole document	1-9
	--	

* Special categories of cited documents: [10]

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IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
17th January 1990	15.02.90
International Searching Authority	**Signature of Authorized Officer**
EUROPEAN PATENT OFFICE	L. ROSSI

III. DOCUMENT CONSIDERED TO BE RELEVANT (CONTINUED FR M THE SECOND SHEET)

Category*	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No
A	DE, A, 2331001 (BAXTER LABORATORIES) 17 January 1974, see the whole document --	1-9
A	EP, A2, 0038134 (WHITLOCK,GERALD DAVID) 21 October 1981, see the whole document --	1-9
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ANNEX TO THE INTERNATIONAL SEARCH REPORT
ON INTERNATIONAL PATENT APPLICATION NO. PCT/GB 89/01228

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This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report.
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